THE PATHOLOGY OF AMANITA PHALLOIDES INTOXICATION.*

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UP to the time of Kobert's publications[1] concerning the active principle of *Amanita phalloides*, little was positively known in regard to the poisons of this fungus, although Boudier,[2] Letellier and Speneux,[3] and Oré[4] had each of them made a chemical analysis of the plant and had extracted certain principles to which the names bulbosin, amanitin, and phalloidin had been respectively given. The ground for criticism of the previous work lay in the imperfect identification of the specimens used by the early investigators, and in a possible admixture of *Amanita muscaria* from which Schmiedeberg and Koppe[5] had obtained the alkaloid muscarin. Kobert's studies in 1891 demonstrated that aqueous and saline extracts of fungi, identified beyond peradventure as *Amanita phalloides* and as the fungus known to be the cause of nearly all the fatal cases of mushroom intoxication, contained a powerful hemolytic substance, dissolving a great variety of corpuscles, his dried material acting upon ox blood, for instance, in a dilution of 1 : 125,000. The discovery of the hemolysin in these fungi was a very great advance in the knowledge of poisonous plants and to its presence in *Amanita phalloides* Kobert naturally attributed the toxic action of the fungus on man. This hemolytic substance, to which Kobert gave the name phallin, was highly unstable, easily destroyed by acids, alcohol, etc., and was rendered inactive by exposure to a temperature of 70° C. He believed it to be a proteid belonging to a class of poisonous substances characterized as "toxalbumins," in which group he placed also the hemolytic poison of spiders (Spinnengift). How such a labile substance,

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[1] *St. Petersb. med. Wchnschr.*, 1891, 16, pp. 463, 471.

[2] Des champignons au pont de vue de leurs caractères usuels, chimiques et toxicologiques, Paris, 1866.

[3] *Ann. d'hyg. publ.*, 1867, 27–28, p. 71.

[4] *Bull. Acad. de méd.*, 1877, 13, p. 350; *Arch. de phys. norm. et path.*, 1877, 2, p. 275.

[5] *Das Muskarin*, Leipzig, 1869.

so susceptible to acid and heat as phallin, could be the active principle in poisoning where cooked fungi were taken into the stomach was never satisfactorily explained by Kobert, nor could all the lesions found in fatal cases in man be justly referred to the action of a blood-laking agent alone.

Nevertheless, despite the objections of such excellent authorities as Kunkel,[1] Bourquelot,[2] and others to the complete acceptance of phallin as the active principle of *Amanita phalloides*, Kobert's observations were really the first of any value in this subject and his views were therefore adopted by the majority of mycologists and found their way into all the standard textbooks. In 1899 Kobert[3] published a second communication upon the poisons of the deadly Amanita in which he stated that later investigations of the plant failed to reveal the constant presence of phallin, and that in addition, the alcoholic extract of the fungi contained a highly poisonous non-hemolytic substance fatal to small animals in minimal doses but not producing any fatty degeneration of the organs, the lesion which is accepted by all writers on this subject, as the most characteristic change in man. This second substance Kobert stated to be an alkaloid, without, however, specifying any alkaloidal reactions to which it responded. The failure of this substance to produce fatty degeneration precluded, according to Kobert, any possibility of its being the active principle. In 1906[4] we were able to confirm Kobert's observations upon the hemolytic activity of aqueous and saline extracts of the fungus in question and later[5] pointed out the fact that heated extracts, no longer hemolytic, were still powerfully toxic to small animals. The fungus must therefore contain another poison, heat-resistant in character, in addition to the blood-laking substance described by Kobert. To this second poison we gave the name Amanita-toxin.

Subsequently, in a chemical investigation of the fungus in the Pharmacological Laboratory, made by Dr. Abel and myself,[6] it was

[1] *Handbuch der Toxikologie*, Jena, 1901, 2, p. 1048.

[2] Article on "Champignons" in Richet's *Dict. de phys.*, Paris, 1898, 3, p. 271.

[3] *Sitzungsb. d. Naturf. Gesellsch.*, 1899, p. 26; Anhang to the *Arch. d. Ver. d. Freund. d. Naturg.*, 1899, 53, zweite Abtheilung.

[4] Ford, *Jour. Infect. Dis.*, 1906, 3, p. 191.

[5] Ford, *Jour. Exper. Med.*, 1906, 8, p. 437.

[6] *Jour. Biol. Chem.*, 1907, 2, p. 273.

shown that on the addition of alcohol to aqueous extracts the hemolysin is precipitated, while the alcohol-soluble fraction contains a substance extremely poisonous to both rabbits and guinea-pigs, identical in its action with the Amanita-toxin already described. This Amanita-toxin we suspect may be the same poison as described by Kobert in his second communication and characterized by him as an alkaloid. It was also shown by us that the hemolysin in *Amanita phalloides* is not a toxalbumin, as stated by Kobert, since all proteid may be removed from it by the use of freshly prepared metaphosphoric acid and by uranyl acetate without appreciable impairment of its hemolytic activity. For the present, at least, we believe that this Amanita-hemolysin, as we shall call it, must be classed at a glucoside, inasmuch as our purest proteid-free hemolytic preparations always reduce Fehling's solution after hydrolysis with mineral acids, and give characteristic tests for pentoses. The extreme lability of this substance and its sensitiveness to the action of heat and acids at once raises the question of its playing any rôle in human intoxications. The observations of Dr. Schlesinger and myself[1] finally upon the chemical properties of Amanita-toxin indicated that this substance is not a proteid or an alkaloid, but either an indol or pyrrol derivative or an aromatic phenol so combined with an amine group that it readily forms an indol or pyrrol ring on fusion. In the last edition of his textbook,[2] Kobert's views have undergone another modification. In this publication he assigns to the fungus three separate poisonous substances, the hemolytic principle, phallin, the alcohol-soluble alkaloid not producing fatty degeneration, and finally a "toxalbumin" present in the alcoholic precipitate of the aqueous extract, allied chemically to the substances thujon and pulegon, and capable like them of causing fatty changes in the internal organs. As far as can be judged from this latest communication, Kobert presents no experimental evidence as to the presence of this third poison in *Amanita phalloides*, assuming it to be present there in order to explain the fatty degeneration seen in fatal cases in man. It therefore becomes necessary to consider the subject more fully from the pathological standpoint, to determine in how

[1] *Jour. Biol. Chem.*, 1907, 3, p. 279.

[2] *Lehrbuch der Intoxikationen*, Stuttgart, zweite Auflage, 1906, 2, p. 614.

far the lesions found in man can be explained by the action of these poisons already demonstrated in the plant, and to inquire into the necessity of hypothecating any other toxic substance.

LESIONS FOUND IN AMANITA PHALLOIDES INTOXICATION IN MAN.

The principal pathological changes in fatal cases of this intoxication were described originally by Maschka[1] over a half-century ago. From a series of seven autopsies he summarized the lesions as follows: (1) Lack of post-mortem ridigity; (2) Widening of the pupils; (3) Failure of the blood to coagulate and a cherry red color: (4) Ecchymoses and hemorrhages in the serous membranes and parenchymatous organs; (5) Dilatation of the bladder with urine. In addition Maschka observed a fatty degeneration of the internal organs, a change not noted by the earlier observers, but failed to regard this lesion with the attention it deserved.

Carayon[2] in 1873, in autopsies upon soldiers who died three days after eating cooked *Amanita phalloides*, noted an inflammation of the walls of the stomach and intestine, a congestion of the liver and kidney, a dark red and fluid condition of the blood, and in one case, a congestion of the meninges, thus confirming Maschka's observations in part. A little later Chouet and Pelissié[3] studied the lesions in two individuals dead in 48 and in 60 hours from the time of ingestion of the fungi. They found the contents of the stomach and intestine bloody, with ecchymoses in the gastric mucosa and in the liver. The meninges also were hyperemic and the pia-mater showed ecchymoses. Possibly the most careful microscopic examination of the organs in this condition was made by Sahli[4] in two cases. The gross lesions were similar to those described by Maschka, and included subpleural and intrapulmonary hemorrhages and a general atrophy of the panniculus adiposus. In addition Sahli found a fatty degeneration of the liver and kidney, the heart, and the diaphragm, as well as of the voluntary muscles, such as the pectorals, deltoid, abdominal, and the tongue. The amount of fat in the liver was so great as to remind him of acute phosphorous poisoning, with

[1] *Vrtljschr. f. d. prakt. Heilk.*, 1855, 46, p. 137.

[2] *Gaz. d. hôp.*, 1873, 46, p. 1115.

[3] *Gaz. hebd. de méd*, 1880, 2d s., 17, p. 68.

[4] Studer, Sahli, und Schärer. *Mitth. der naturfors. Gesellsch.*, 1885, p. 81.

which phalloides intoxication has ever since been compared. In the alimentary tract the mucosa of the stomach and intestine were much congested, the Peyers patches and solitary follicles swollen. In 1886 Handford[1] in an autopsy on a man dead three days after being poisoned, found ecchymoses on the pleural and pericardial surfaces, a congestion of the mucous membrane of the stomach, with capillary hemorrhages, and excoriations (ulcers), and a general congestion of the intestine. The kidney was anemic and the liver fatty.

Tappeiner[2] subsequently had the opportunity of carefully studying the bodies of two children dead from eating *Amanita phalloides*. In one case there were a number of small punctiform hemorrhages beneath the skin and in both cases the stomach and intestines showed the characteristic injection of the mucous membrane with small hemorrhages, and a swelling of the solitary follicles, the Peyers patches, and the mesenteric lymphatics. Minute hemorrhages were also visible on the surface of the spleen, the kidney, and the liver, and on section of these organs similar changes were apparent. In one of the cases in which jaundice was present during life, the liver was yellowish green in color and brittle in consistency. On microscopic examination, the liver and kidney revealed fatty degeneration and infiltration of the cells, with infarcts, like the changes seen in phosphorous poisoning, while similar infarcts in the heart muscle showed evidences of blood destruction. The heart muscle also was fatty. The percentage of fat in the liver was estimated in these two cases by Tappeiner, who found 53.6 per cent in one case and 68.9 per cent in the other. The fat content of the liver under normal circumstances varies from 8 to 25 per cent (Perls), and in these cases the percentage of fat is thus equal to that found in phosphorous poisoning, usually from 50 to 70. This percentage of fat obtained by Tappeiner was confirmed by Thiemich[3] who estimated the amount in the liver in one case at from 69.1 to 69.3 per cent and in another at from 73.1 to 73.5 per cent.

The lack of post-mortem rigidity noted by Maschka and by Sahli and the widening of the pupils mentioned by Maschka were confirmed

[1] *Lancet*, 1886, 2, p. 1018.

[2] *Münch. med. Wchnschr.*, 1895, 42, p. 133

[3] *Deutsche med. Wchnschr.*, 1898, 24, p. 760.

by Moers,[1] whose three cases were especially marked by the extent and distribution of the hemorrhages. These were present in the stomach and intestine, on the surface and in the substance of the liver and kidney, in the heart, the pericardium, the external coat of the aorta, and in two cases in the ovary, and in the brain. In these cases the various organs were tested for arsenic, phosphorous, and muscarin, in the absence of which substances the lesions found could only be attributed to the poisons of the fungus itself. Finally Plowwright[2] has recently reported for the second time an autopsy upon a boy of 12 years, who died five days after eating a third of the pileus of an uncooked specimen. In this case the gastric mucosa was much injected and softened, there were gangrenous spots on the mucous membrane of the intestine, and a general peritonitis. In another autopsy reported at the same time, the mucous membrane of the colon was studded with pearly white tubercles which turned out to be the much enlarged solitary glands.

From these various observations, it is evident that the most important lesions in man consist of the intestinal ulceration, the swelling of the lymphatic tissue, the general congestion of all the organs, the widespread hemorrhages, the necrosis and the fatty degeneration, especially marked in the liver and kidney. This picture is not one of a hemolytic intoxication, such as is that produced by the ingestion of *Helvella esculenta*, whose active principle, helvellic acid, is a blood-laking poison resistant to the action of heat and the digestive juices. The two most important signs of blood destruction, hemoglobinurea and increased pigmentation, particularly of the spleen, while not entirely lacking, are so little in evidence as to escape mention. The evidence at hand points rather to the action of a profound cellular or protoplasmic poison, acting upon the cells of the parenchymatous organs, and resulting in an extensive deposition of fat, and also causing profuse hemorrhages, probably by a similar destructive action upon the cells of the capillary endothelium. The clinical data available lead one to a like conclusion, since vomiting and diarrhea, with exhausting hemorrhage from the mucous membranes and profound prostration are among the early symptoms, followed in the severe cases by

[1] *Ztschr. f. med. Beamt*, 1903, 16, p. 412.

[2] *Brit. Med. Jour.*, 1905, 2, p. 541.

rapid loss of weight, jaundice, coma, and death. No mention is made of the presence of blood pigment in the urine, and in one case of which I have personal knowledge, the urine was free from color. While the pathological changes, as described, and the clinical symptoms both tend to rule out the action of the hemolysin in alimentary intoxications, this point cannot be definitely settled until a more careful study can be made of cases in man, with the especial end in view of determining the extent to which blood destruction has taken place, and how far it can be deemed responsible for the death of the patient. Some of the lesions mentioned, such as the fluid condition of the blood, seem to indicate, moreover, a certain amount of blood destruction. Since the characteristic lesions are the intestinal ulcers, the fatty degeneration of the organs, and the hemorrhages, it is necessary to determine whether these can be produced experimentally in animals, and to which fraction of the fungus they can in all probability be assigned. It is of especial importance to determine which portion of the fungus can produce fatty degeneration, since Kobert has stated that his alcohol-soluble alkaloid is incapable of causing such a change and has hypothecated a third poison, a toxalbumin like thujon or pulegon, in order to explain this excessive fat production in man.

LESIONS PRODUCED BY THE WHOLE EXTRACT

The action of the aqueous or saline extract of *Amanita phalloides* is the same upon both rabbits and guinea-pigs, the amount of the poison necessary for a fatal dose depending largely upon the weight of the animal selected. The animals usually die after the lapse of four to six days, but may succumb to large doses within 24 or 48 hours. At autopsy an extensive gelatinous edema is found in the subcutaneous tissues at the site of inoculation, the edematous tissue exuding a thin reddish fluid on pressure. Minute hemorrhagic areas are visible everywhere in the neighboring fascia and muscular tissue, while the adjacent lymphatic glands are swollen and hemorrhagic. On opening the abdominal cavity, the blood vessels are found much injected, but the most noticeable phenomenon is the presence of the widespread hemorrhages. These vary in size from small ecchymoses on the surface of the liver and kidney to considerable collections of blood between the layers of the peritoneum. Both liver and kidney

are congested, showing on section a general dilatation of the blood vessels with occasional minute areas of hemorrhage. The adrenals exhibit this condition of congestion and hemorrhage to a marked degree, while the lymphatic glands in the abdominal cavity show similar changes. In female animals, both ovaries and uterus may show areas of hemorrhage, and in one case—a pregnant rabbit—there was considerable free blood in the ammotic sac. The bladder is usually filled with urine which is deeply blood stained. On centrifugation no intact blood corpuscles are present, the urine retaining its reddish hue, a result which points to a true hemoglobinurea. In the pleural cavity, hemorrhages are found between the folds of the pleura and minute ecchymoses on the surface of the lung. Rarely more extensive hemorrhages are produced, an entire lobe of the lung or a considerable portion of it being occupied by the extravasated blood. The heart is in complete diastole, the blood in the heart and in the larger vessels remaining fluid or partially coagulated. The meninges of both spinal cord and brain show little areas of bleeding and on section of these organs small punctiform hemorrhages can be made out. The contents of the stomach and intestine are often blood stained, minute ulcers in the mucosa of the bowel, with extravasated blood in the base of the ulcers, indicating the source of the hemorrhage.

Microscopic examination of the tissues and organs reveals constant and fairly characteristic changes. The connective and muscular tissue is much swollen, the muscle fibers show hyaline degeneration and extravasated blood corpuscles can be seen near the blood vessels and between the muscle bands. The lymphatic glands, both those from the subcutaneous tissues and those in the abdominal cavity, are congested and hemorrhagic, the lymph cells are necrotic with pyknotic nuclei, and there is a general increase in pigment. In the spleen there is always considerable extravasated blood, but the prominent change is the very great increase of blood pigment, while the cells of the splenic pulp are necrotic with pyknotic nuclei. In the liver in addition to the congestion of the vessels, many blood corpuscles lie free in the inter- and intra-lobular spaces and between the hepatic cells. Rarely a direct connection can be traced between these areas of extravasated blood and some one of the smaller capillaries,

where a break in the lumen of the vessels allows the corpuscles to escape. The nuclei of the capillary endothelium are pyknotic, the liver cells show necrosis, and there is a general increase of pigmentation. In the kidney there is a uniform condition of congestion and hemorrhage, the kidney cells are shrunken from the basement membrane and are the seat of hyaline degeneration. The lungs show extensive vascular dilatation with many free blood corpuscles and an excess of blood pigment. The muscle fibers of the heart exhibit hyaline degeneration, nuclear vacuolation, and at times almost complete destruction leading to the appearance of small areas of focal necrosis. Finally there is in all the organs evidence of fatty degeneration, this fat being widely distributed, especially in the cells of the liver and kidney. The pathological changes found in animals thus inoculated with the whole extract of this fungus consist mainly of intestinal ulcers, hemorrhage, necrosis and fatty degeneration, a laky condition of the blood, hemoglobinurea, and wide deposition of blood pigment pointing to an extensive blood destruction. While evidence of all these changes can be secured in animals dying at various intervals, their extent varies considerably. Thus large doses of the whole extract and doses of freshly prepared material produce a more fluid condition of the blood, more pronounced hemoglobinurea and a greater increase of free blood pigment than do small doses, more nearly approaching a minimum fatal dose, or older extracts. With large doses, a greater amount of poisonous substance is injected than is necessary to kill the animal and the excess of hemolysin can exert its destructive effect upon the corpuscles. With the freshly prepared extracts, the hemolysin is more active and smaller quantities serve for a lethal dose than with the older extracts, where the hemolysin can be shown by test-tube experiment to have deteriorated considerably in strength. Changes due to blood-laking are thus naturally more pronounced. By heating the whole extracts to 65°–70° C. for one-half hour, the hemolysin is destroyed, yet the solutions do not lose their toxicity, owing to the presence of the heat-resistant toxin. By inoculating feeble guinea-pigs, with small doses of the fresh extract an acute death can sometimes be brought about apparently by the action of this toxin, since in these cases but little evidence of intra-corporeal blood-laking can be made out.

It is manifest that when we are employing the whole extract, we are inoculating into the test animals a mixture of poisons, the relative amount of which depends upon the age of the preparation, while the lesions produced in the inoculated animals vary with the toxicity of each ingredient and the extent to which it is present in the dose administered. Let us now try to determine what lesions can be produced in animals by the inoculation of the different poisons of the plant, freed from each other, as far as possible, by chemical means

LESIONS PRODUCED BY THE AMANITA-TOXIN.

The alcoholic extract of *Amanita phalloides*, free from the hemolytic glucoside, is extremely poisonous to both rabbits and guinea-pigs and is identical in its action with extracts of the fungus heated to 70° C. and containing the Amanita-toxin previously described. With large doses the animals die acutely, often within 24 or 48 hours, while with lesser amounts death occurs usually after the lapse of six or eight days. At autopsy, there is little or no change at the site of inoculation, except a slight edema, which may be obviated by careful neutralization of the extract. Rarely there is a considerable injection of the blood vessels at this area and minute points of ecchymoses may be found scattered through the adjoining facia and muscles. The lymphatic glands sometimes present no change, but are more frequently congested and hemorrhagic. In the abdominal cavity the vascular changes predominate, ecchymoses and punctiform hemorrhages showing on the surface of the internal organs, with extravasated blood in the mesentery. The organs are pale and the liver especially looks fatty. The adrenals are sometimes normal in appearance but frequently show a condition of extreme congestion and hemorrhage. The bladder is filled with urine, always straw colored and free from blood pigment. In the pleural cavity, small flecks of hemorrhage are visible on the surface of the lungs, and rarely large amounts of blood are found between the layers of the pleura. The heart is widely dilated, the blood always firmly clotted, the same condition holding true for the larger vessels. Occasionally small petechiae are seen on the pericardial surfaces. The spinal cord and brain show no especial changes. In the walls of the stomach and intestine are often found minute ulcers, and there is frequently

dried blood in the contents of the bowel. Careful search nearly always reveals the source of this blood.

Microscopically the changes found in the tissues and organs, relate chiefly to necrosis, fatty degeneration and to hemorrhage. The voluntary and cardiac muscles show no particular edema, but many free blood corpuscles outside the smaller vessels and considerable hyaline degeneration. In the lymphatic glands there is a congestion of the vessels and some extravasation of blood, while the lymph cells show necrosis. There is no special increase of pigment. In the spleen the pigment is increased slightly but not markedly, but there is considerable extravasated blood and the cells of the splenic pulp are necrotic. In both liver and kidney there is an extraordinary deposition of fat. This is present in fine granules or in coarse globules within the cells, and so extensive is this deposition that it seems as if almost every cell had undergone this fatty change. In some animals the liver appears almost entirely transformed into fat. Of especial interest is the presence of fat in the cells of the capillary endothelium, suggesting that the various hemorrhages may be due to a rupture of these small vessels consequent upon a fatty degeneration.

Similarly in the kidney, there is an extreme fatty degeneration of the cells of the glomeruli and of the various tubules, as well as in the endothelial lining of the capillaries. In the lungs, the microscopic changes are chiefly vascular, showing a general dilatation of the vessels and an extravasation of the blood. There is no special increase of pigment in this organ. The gross lesions produced by the Amanita-toxin represent to only a partial degree, therefore, the lesions produced by the whole extract. There is no edematous swelling at the site of inoculation, and no hemoglobinurea. The hemorrhages are by no means as extensive as after inoculation of the whole extract, but they are constantly present and may occur in any situation. Small ulcers may be present in both stomach and intestine. Microscopically the excessive pigmentation of the spleen is absent and the increase of pigment in the lymph glands and in the lungs is less marked. The necrosis and fatty degeneration are present to a greater degree possibly, but this lesion is more easily brought out in these tissues than in the tissues of animals dead of both toxin and hemolysin, since the greater deposition of pigment

and the more extensive escape of blood corpuscles in the latter case may to a certain extent obscure the fatty change. Moreover, but one poison is here administered and larger amounts of this single poison are necessary for a fatal dose than when a poisonous hemolysin is also present, in consequence of which the lesions due to this substance would naturally predominate. In some cases indeed the fatty degeneration seems to be the only lesion present, the evidence of hemorrhage being very slight. Finally the solutions containing the toxin can be heated to between 70° and 80° C. without losing their activity.

LESIONS PRODUCED BY THE AMANITA-HEMOLYSIN.

The Amanita-hemolysin contained in the alcohol precipitate of extracts of the fungi is poisonous to animals but by no means in the same degree as are solutions containing the toxin alone, since the doses required to kill either rabbits or guinea-pigs are much larger. Animals may die acutely in 48 hours from these large doses, but usually death ensues at a later period, possibly after 10 or 12 days. The hemolysin is essentially not so acute a poison as the toxin. At autopsy the most marked leison is the swelling and edema at the site of inoculation with the production of a large amount of fluid deeply stained by blood pigment. The glandular apparatus shows little change microscopically. In the abdominal cavity there is an exudate of blood-stained fluid with a general congestion of the blood vessels, and frequent hemorrhages. The bladder is full of urine, stained dark red by free blood pigment. The heart is dilated and full of laked and fluid blood which fails to clot on exposure to the air. Similar fluid blood occupies the larger vessels. There is no special lesion in the lungs.

On microscopic examination, the most marked change is the great increase of pigment. The spleen is loaded with this substance which is also much increased in the lymphatic glands and in the liver and lungs.

There is some increase of fat in the various organs but this is not present to any considerable extent. There is some extravasation of blood.

The lesions in animals poisoned with this portion of the fungus,

are due to the blood-laking properties of the hemolysin and the whole picture is one of a hemolytic intoxication. The two most characteristic signs of such an intoxication, hemoglobinurea and pigmentation of the spleen, are present to an extreme degree. If extracts containing the hemolysin alone, free from the toxin, be heated to 70° C. for half an hour they lose their blood-laking properties and then may be given in large quantity to animals without injurious effect. Sometimes a little edema develops at the site of inoculation, but the fluid is not stained with hemoglobin and if the extract be made neutral before administration this edema does not develop. It may be due to the irritating action of some acid present in the original aqueous extract of the plant and precipitated by ethyl alcohol.

SUMMARY OF PATHOLOGICAL CHANGES.

The principal lesions found in phalloides intoxications in man can be produced in animals by the administration of alcoholic extracts containing the toxin, freed from the hemolytic glucoside. These lesions are the hemorrhages, the cellular necroses, the fatty degeneration, and the gastric and intestinal ulcers. They may also be produced by aqueous extracts of the plant containing both toxin and hemolysin, but with the whole extract the hemorrhages are far more extensive, and there is in addition a true hemoglobinurea and a great increase in the pigmentation of the organs, especially the spleen. The Amanita-hemolysin free from toxin reproduces these latter lesions but not the important lesions seen in man. The toxin alone reproduces none of the phenomena of a hemolytic intoxication. The sensitiveness of the Amanita-hemolysin to heat and acids, and the resistance of the Amanita-toxin to these agencies, would indicate that the hemolysin plays but an unimportant rôle in alimentary intoxications where the cooked fungi are introduced into the stomach, in conformity with which is the fact that in man the lesions generally described point to the action of a protoplasmic poison, and not to a hemolytic agent. The action of the hemolysin in man, cannot be entirely ruled out on pathological grounds, since our knowledge of the pathology of human intoxications is far too meager to permit positive conclusions as to the character of the microscopic changes. It has also been shown that hemolytic substances are present in two

species of fungi usually believed to be edible, *Amanita solitaria*, and *Amanita rubescens*,[1] and that certain specimens of *Amanita phalloides* typical in other respects are devoid of hemolytic activity. In the majority of instances, however, and in all cases where chemical examination of the plant has revealed the presence of a powerful toxin, *Amanita phalloides* contains an abundant and extremely active hemolysin, which is present regardless of the locality from which the fungi are collected. In the ordinary method of cooking fungi for the table this hemolysin may not be entirely destroyed and it may be introduced into the stomach in such large quantities as to escape the action of the gastric and intestinal juices, especially as if these should be deficient in acid or in ferments. Furthermore, in many fatal cases of mushroom intoxication the fungi are eaten improperly cooked and even raw, as for instance by little children playing in the woods. For these reasons it is impossible to eliminate the action of the Amanita-hemolysin in man, although it is evident that the Amanita-toxin plays the more important rôle of the two substances. Just what relation the alcohol-soluble alkaloid described by Kobert bears to our alcohol-soluble Amanita-toxin is not clear since he expressly stated that his substance does not produce any fatty degeneration. The Amanita-toxin, however, produces this fatty change to a marked extent, and is undoubtedly responsible for this lesion in man. On this account there is no need to hypothecate a third poisonous substance, a toxalbumin like thujon and pulegon to explain the fatty degeneration of liver and kidney so characteristic of human intoxications.

ALIMENTARY ADMINISTRATION OF AMANITA PHALLOIDES IN RABBITS.

In an attempt to reproduce in animals the conditions of intoxication by this fungus in man, an attempt has been made to poison rabbits through the administration of various extracts of the fungus by mouth. A small rubber tube was passed into the stomach and both hemolysin and toxin given in large quantities. To our great surprise it was found that these animals are absolutely resistant to the action of the poisons of this fungus in this method of administration. Both hemolysin and toxin are quite inocuous. This was of special interest in regard to

[1] Ford, *Jour. Infect. Dis.*, 1907, 4, p. 434.

the toxin which we know is not destroyed by the digestive ferments in man, and on several occasions highly toxic solutions were given without injurious effects. As much as 10 c.c. of a toxin in which $\frac{1}{4}$ c.c. would kill rabbits on subcutaneous inoculation was administered to a number of rabbits, but there seemed to be no harmful results. Extracts of other Amanitas, *Amanita solitaria* and *Amanita rubescens*, containing strong hemolysins were also given to rabbits by mouth but no injurious effect was seen. Whether it is an infallible rule that rabbits can take these poisons into their stomach without disastrous consequences, or the mechanism by means of which the poison is destroyed is in their alimentary canal is not known. It would, however, be remarkable in the economy of nature, if such an abundant plant as the *Amanita phalloides* could not be used as food by some animals, and possibly it can be so utilized by the herbivora. We know that both dogs and cats are poisoned by the cooked fungi in the same degree as are human beings.

APPLICATION OF THESE FACTS TO SERUM-THERAPY.

In our early work on the production of immunity in animals, by the administration of gradually increasing doses of extracts of the fungus, the claim of Kobert that the hemolysin in this plant is the active principle was believed to have been established. It was not found difficult to produce an active immunity in rabbits in which the animals would withstand the inoculation of two or three fatal doses, and the serum from these animals was highly antihemolytic, in a dilution of 1:1,000, or as high as 1:5,000. When tested upon animals it was also antitoxic to a limited degree, 1 c.c. of the serum neutralizing two or three times a minimum fatal dose. While the strongest serum obtainable neutralized but six or seven times a fatal dose, this serum contained such a powerful antihemolysin that we were led to believe that a serum from large animals more highly immunized might be of therapeutic use. The antihemolytic power of the serum *in vitro* was looked upon as a valuable index in the estimation of its antitoxic power.

In attempting to produce a higher degree of immunity, however, the mortality among the animals was much increased, the cause of this phenomenon not being apparent at that time. A similar experience with larger animals such as goats and horses indicated that there

were other factors to be considered than the hemolytic activity of the extracts and the amount of antihemolysin the animals produced. The most valuable serum obtained was made by Dr. Kinyoun from a horse, at the Mulford Laboratories at Glenolden, Pa. Although the anititoxic power of this serum was so low as to be of little practical value, ¼ c.c. of the serum neutralizing a toxic dose of the fungus for a 500-gram guinea-pig, yet this serum contained a strong and permanent antihemolysin operative in a dilution of 1:1,000, using as an index that quantity of hemolysin which will dissolve 1 c.c. of a 5 per cent blood suspension. It has now been shown by the observations of Kobert and by our own work that the hemolysin in the fungi is accompanied by this powerful toxin, which may play the more important rôle in man. Experiments were therefore instituted to determine the extent to which animals can be immunized to these two substances completely separated by chemical means. With the pure hemolysin freed from toxin, rabbits can easily be immunized. There is at first a slight loss in weight, and some edema at the site of inoculation. The animals soon recover their weight, the subcutaneous edema disappears, and after five or six doses, fairly large quantities of the hemolysin can be administered. The serum from these immunized animals is antihemolytic, a strength of 1:1,000 being obtained after half a dozen doses. With the pure toxin, immunization is attended with a high mortality and the serum produced by these animals successfully immunized has but a limited quantity of antitoxin. Active immunity up to the administration of two or three multiples of a fatal dose is not difficult, but beyond this point the animals die just as they do with the whole extracts. At no time has a stronger serum been obtained with this pure toxin than with the mixture of toxin and hemolysin originally employed. We are therefore confronted with this condition. It is possible to make an antibody for the hemolysin in the fungus and such an antihemolysin would have practical value to just that extent to which the hemolysin acts in man. Although attempts are still being made to prepare an antitoxic serum, for that portion of the fungus which plays the more important rôle in man, the results thus far obtained have not been accompanied with a sufficient degree of success to justify any attempt to make a practical application of our work. The demonstration by Dr. Abel and myself

that the Amanita-hemolysin, for which we have repeatedly made an antihemolytic serum, is a glucoside is not without theoretical interest, both in regard to current opinion on this subject and with reference to the possibility of making antibodies to other poisons of a glucosidal nature.

CONCLUSIONS.

1. The lesions found in fatal cases of *Amanita phalloides* intoxication in man consist of ulcers in the stomach and intestines, hemorrhages in the serous membranes and parenchymatous organs, necrosis of various cells, and fatty degeneration, especially advanced in the liver and kidney.

2. The lesions produced by the whole extract of the fungus consist of gastric and intestinal ulcers, hemorrhages, necrosis of cells, fatty degeneration, hemoglobinurea, and pigmentation of the spleen and other organs.

3. The lesions produced by the Amanita-toxin consist of gastric and intestinal ulcers, hemorrhages, necrosis, and fatty degeneration, and to a certain extent they approximate the lesions seen in man.

4. The Amanita-hemolysin acts upon animals by virtue of its blood-laking properties, producing the hemoglobinurea and the pigmentation of the spleen characteristic of hemolytic intoxications.

5. The Amanita-toxin is probably the more active principle in alimentary intoxications in man since with it we can produce experimentally the lesions usually found. The action of the hemolysin cannot be ruled out in cases where large quantities of insufficiently cooked fungi are consumed or where the fungus is eaten raw.

6. Inasmuch as the Amanita-toxin will produce fatty degenerations in a marked degree, there is no need to assume the presence of a hypothetical toxalbumin like thujon or pulegon in order to explain the fatty character of the lesions.